HYDRATION AND GROWTH.*

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(*Read April 25, 1919.*)

The studies described in the present paper are based upon the results of three methods of observation and experimentation, as follows:

(*a*) A comprehensive series of measurements of the variations in volume of stems, leaves and fruits have been made in which the course of growth and variations in rate have been correlated with variations in such environmental factors as temperature, humidity, water supply, etc. The records include the entire developmental period of many stems extending in some cases over a period of two years. A paper descriptive of some of this work was presented before this Society three years ago and was printed in the *Proceedings* for 1917.

(*b*) Attention has been directed to the determination of the composition of living matter and the manner in which its components are united or mixed in the cell. Series of analyses arranged to show not only the general character of the cell contents, but also the seasonal and developmental changes in plants have been made, in connection with the comprehensive work of Dr. H. A. Spoehr upon carbohydrate metabolism (now in press).

(*c*) Measurements of the hydration reactions of tracts of living cell-masses have been compared with the reactions of sections of plates of colloids made up in simulation of the composition of plants show that the water relations of the living material of the higher plants are those of a colloidal mixture consisting predominantly of pentosans, of a lesser proportion of albumin, albumin derivatives and amino-compounds and of a minor proportion of lipins, with an inevitable small amount of salts.

The components of these three groups are not mutually inter-diffusible to any extent and hence in my colloidal preparations, as

* Abstract prepared by author from a lengthy manuscript.

well as in the protoplast, must be considered as simply mixed in an intimate manner in interlocking meshworks, foams or whatever interpretations may be given to the structures revealed by cytological technique. Living matter made up in this manner may be miscible, or immiscible, according to the nature of the pentosan or protein entering into its composition. It is obvious that the protoplasm in which the carbohydrate element is like agar would not be "soluble," while a mixture composed largely of gum arabic would go readily into suspension. Hydration as used in this paper denotes the union of water with the molecules or aggregate of molecules of substances in a colloidal condition inclusive of the action by which first definite proportions are taken up in so-called chemical combination, and also of the indefinite absorption combinations.

Growth may be defined as consisting chiefly in the hydration of colloidal material in a living condition generally accompanied by accretions to the main components as described above, although not actually necessary for the conception as the ground or fundamental structure of protoplasm might be increased by rearrangements of material already included. The later stages of increase in volume of a protoplast are coincident with the formation of denser permanent structures having the effect of increasing the relative dry weight with age or approaching maturity, a procedure which may be taken to be universal in the tissues of the higher animals, according to the researches of Donaldson, Hatai and others.

This too is currently assumed to be the case in the tissues and organs of the higher plants, but my own results include a type of growth in which this is not the case. Succulents of all kinds produce stems or leaves in which the percentage of dry weight of small young organs such as the joints of cacti, leaves of *Mesembryanthemum,* and fruits of the melon or berry type, is greater than that of mature organs or members exemplifying a category of growth especially interesting in the present connection. Succulence is due to the hypertrophy or exaggerated growth of cells in which the hexoses have been converted into pentosans, initially as a result of low water contents of the cells, the pentosans having a high capacity for water which is exerted during the remainder of the life of the cell, and it is in such organs that the relative dry weight does not increase with age.

The living matter of the plant cell includes pentosans which are to be taken as weak acids and which show only slight dissociation, and of albumins or amino-compounds, which undergo a greater hydration with increasing concentration of hydrogen ions. Hydration of the carbohydrate or pentosan constituent or protoplasm is affected in the reverse manner. The actual rate of growth of any protoplast would therefore be a resultant of the opposing action of pentosans and of albumins.

Swelling, hydration or growth of bio-colloidal masses is not however simply a matter of hydrogen ion concentration, as we have found that the hydration and swelling of pentosan-protein colloids simulating protoplasm, of tissues of living plants and of dried cell-masses, is facilitated by the amino-compounds which dissociate as bases. The greatest increases occur in concentrations of 0.01 *M* to 0.001 *M* of such substances as alanin, asparagine, glycocoll, phenylalanin, and ethylamine. Concurrent accelerations of growth resulting in increased dry weight and greater volume are reported by many experimenters using such substances in culture solutions. It is highly probable that bases (cations) may have some effect on hydration or growth, a matter yet to be tested.[1]

The essential feature of an idealized growth is the accretion or addition of water and material to the mass of colloid constituting the cell. The actual mechanism of incorporation is not easily delineated. If protoplasm consisted of a system of colloidal structures such as those of the pentosans and the proteins interwoven, but not diffusing into each other, the more solid material which lowers the surface tension to the greatest extent, having the least attraction for water-molecules, would tend to usurp the position of the surface layer. Furthermore, the solid phase, whether it be in the form of globules or in the continuous element, would tend to increase and crowd together with a lessening of the more liquid phase. This would imply that when gelatine in small proportion is mixed with agar or starch paste in the larger proportion that the carbohydrate would form both the colloidal framework or mesh, as well as the external layer

[1] See MacDougal and Spoehr, "The Effect of Organic Acids and their Amino-Compounds on the Hydration of Agar and on a Biocolloid," *Proc. Soc. Exper. Biol. and Med.*, 16: 33. 1918.

of the mass. In actual practice the mixtures are solidified too quickly for this to occur.

The separate colloidal masses where they do exist have, of course, definite boundary layers, as are formed wherever two colloidal phases meet. Protoplasm may not be regarded however as altogether a mechanical admixture of minute strands of material of different composition. Much of it, including the more fluid portions, must consist of molecules of carbohydrates, proteins, salts and even lipins aggregated to form submicrons in the disperse phase or in the denser more solid fibers, mesh or honeycomb of the structure. The external layer formed might well be in a sense a mosaic, but it is to be noted that no actual proof of such a condition is at hand. Both absorption or imbibition and osmosis including differentiated diffusions would be affected by the composition and relations of the two phases of the colloids in this outer layer, and it seems highly probable that an adequate interpretation of permeability will be obtained by a study of these features. Meanwhile no general agreement as to the nature of the "membrane" or its action is to be expected until many widely current assumptions are discarded. The external layer of a protoplasmic unit is in every case a product of the surface energy of the mass or systems of living material internal to it and of the medium and it has no other permanent or morphological value. Its constitution must necessarily vary widely as does that of the living protoplasm.

The experimental studies described in the present paper have been devoted chiefly to the action of protoplasmic masses in which the pentosans in colloidal combination with proteins and salts determine the volume or mass of the living material of the cell. The other soluble carbohydrates, including the hexoses, sucrose, dextrose, do not occur in the cell in such concentrations as to affect the enlargement of the protoplasmic mass directly, but in the vacuoles they may exert an osmotic effect additive to that of the amino-acids which may accumulate in these cavities. It is to the osmotic activity of these substances in the vacuoles that turgidity is due and a by no means unimportant part in the maintenance of the rigidity of organs and other features is to be ascribed to these turgor stresses and tensions. The inadequacy of osmotic phenomena and of the concep-

tion of the semi-permeable membrane to provide a mechanism for the translocation of complex material from cell to cell, and the incorporation of new material in a growing mass has long been recognized. That osmotic pressure however may play an important part in the enlargement of the plant cell may well be concluded from the fact that in the stage following the initial swelling of the embryonic cell, a large share of the increase in volume is due to the increase of the vacuoles. It would be a mistake to conclude that the vacuole is simply a sac charged with electrolytes, as these cavities invariably hold proteins and carbohydrates in a colloidal condition in which the degree of dispersion may vary widely, but still absorb water. A correct estimation of the manner in which osmosis and imbibition interlock in growth is one of the tasks demanding the immediate attention of the physiologist.

The Effect of Organic Acids and their Amino-Compounds on Growth.

The accelerating effects of acids or of the hydrogen ion concentration on hydration of proteins is well known, and something of the retarding effect on the swelling of mucilages or pentosans have been described in recent papers.

The effect of bases (cations) on these processes has not yet been measured, nor has the method by which the amino-compounds act in this matter been determined.

The hydrogen ion concentration of the liquids in the plant cell remains constant within limits and in succulents examined by Jenney Hempel may be expressed by P_h 3.9–7, as determined by electrometer and titration methods. The presence of the acids of which malic is the more abundant, and its combinations with such bases as potassium, sodium, calcium, magnesium, iron and aluminium makes a "buffer" by which the degree of dissociation is controlled.

In addition to this comparative stability or narrow range of the concentration of hydrogen ions, amino-compounds are invariably present, and their relative amount probably varies but little.

A series of tests were planned in which a comparison would be made possible between the action of some of the commoner organic acids and of their amino-compounds.

Two groups were chosen for the tests. Succinic acid and amino-succinic or aspartic, which are dibasic, and its amide as noted above which is monobasic, and acetic acid and amino-acetic or glycocoll, which are monobasic. Sections of plates of agar, gelatine, agar-gelatine, agar-protein and other mixtures were used. Swellings were carried out in the equable temperature chambers of the Coastal Laboratory at 15–16° C. A tabulation of the principal results is given on the following page.

The two organic acids, succinic and acetic, are seen to exert the classical effect on gelatine, the greatest hydration taking place in the higher concentrations, the effect decreasing with dilution until at 0.0004 *N* the swelling in acetic acid was scarcely greater than in distilled water. At 0.0004 *M* however the dibasic succinic acid showed a swelling less than that in distilled water, a fact which suggests a rapid solution or dispersion from the surfaces of the sections and alterations of viscosity in the mass.

Mixtures of agar (8) and gelatine (2 parts) were now tested, and the hydration in succinic at 0.00008 *M* was but 1030 per cent. as compared with 1684 per cent. in water, while acetic acid was slightly higher, 1167 per cent. A similar statement would hold for the action of these acids on agar, and for agar-protein, the hydration of water along being reached more nearly than in the agar-gelatine sections.

When we now turn to amino-succinic or aspartic acid and amino-acetic acid or glycocoll, some new relations are uncovered.

The aspartic acid (amino-succinic) appeared to exercise a notable influence on the hydration of agar within the range of its solubility. When more than this was added to the water used for solution a swelling in excess of the expectancy resulted. It was also seen that the surface of the liquid became covered with thin crystals. In all probability the solution or dispersion of some agar into the water resulted in the displacement of some of the acid with the result that the sections were actually hydrated from a solution less concentrated, giving a swelling in excess of the expectancy.

Asparagin was now applied in a series of concentrations to sections of agar of the above swelling capacity in water and it was found that hydration was actually increased or accelerated by the

HYDRATION OF AGAR, GELATINE, AGAR-GELATINE AND AGAR-OAT-PROTEIN IN ORGANIC ACIDS AND THEIR AMINO-COMPOUNDS AT 16–17° C. EXPANSION IN PERCENTAGES OF DRIED THICKNESS.

Concentration.	Succinic Acid Mol.	Aspartic Acid Mol.	Asparagin Mol.	Acetic Acid Mol.	Glycocoll Mol.
		Agar.			
0.3	....	....	....	....	1950%
0.5	....	....	....	1060%	2804
0.1	....	1000%	2260%	1333	....
0.05	1091%	827	2308	1433	....
0.01	1273	1270	2365	1560	2964
0.002	1600	1400	2440	1790	3166
0.0004	1750	1788	2720	1955	2605
0.00008	2528	2080	3250	2640	....
Water	Average:	2600%			
		Gelatine.			
0.1	....	....	....	....	....
0.05	1200%	1500%	320%	952%	370%
0.01	700	1033	480	714	....
0.002	500	380	500	690	360
0.0004	433	340	467	643	360
Water	Average:	600%			
		Agar 8—Gelatine 2 Parts.			
0.5	....	....	....	....	....
0.1	....	....	....	850%	....
0.05	716%	910%	1485%	850	1233%
0.01	860	1017	1574	900	1960
0.002	917	1295	1608	922	1767
0.0004	1000	1667	1383	1117	1420
0.00008	1030	1786	1383	1167	1484
Water	Average:	1684%			
		Agar 8—Oat-protein 2 Parts.			
0.5	....	....	....	500%	....
0.1	....	....	....	809	....
0.05	700%	855%	1867%	1090	1983%
0.01	864	900	2455	1255	2340
0.002	909	1670	2523	1738	3050
0.0004	1136	2500	2675	2238	3000
0.00008	2330	3050	2600	2480	....
Water	Average:	2365%			

presence of this substance. That this result did not simply appear by faulty comparisons was shown by the following replacement test.

A trio of sections which had been swelled in distilled water to a

total of 2630 per cent. and which had stood in the solution without any perceptible change for a few hours after the close of the test was now treated to a 0.01 M asparagin solution. The mechanical disturbance which might result from changing the liquid in the dishes was minimized by fractionization. About one third of the water was removed then the level was raised by the addition of asparagin solution and this was repeated about a half dozen times, the final result being a solution which was diluted slightly below hundredth molecular. A slow expansion began at once which continued for about 20 hours, which raised the total hydration of these sections to 2890 per cent., an increase of 230 per cent., due to the action of the asparagin on sections which had undoubtedly been reduced in mass somewhat by solution from the surfaces.

The presence of asparagin in the water in which swelling of gelatine was carried out, produced an uncertain effect by reason of supposed solution or dispersion of the gel. Neither can much be said concerning its action on agar-gelatine mixtures, except that the results show a maximum at 0.01 M.

When the asparagin is applied to mixtures in which the gelatine is replaced by an albumin, the results included some special reactions. Plates of agar and oat-protein were made up to contain 8 parts of the first and 2 of the last, coming down to a thickness of 0.22–0.23 mm. These swelled at 17° C. to the proportions shown in the table, which in some cases exceeded that in water. The swelling in concentrations as high as 0.01 M were but little below that in water.

Glycocoll has been used in many cultural tests with plants and various interpretations have been placed on its accelerative influence on growth, and its influence on swelling therefore has an unusual interest.

Thin sections of agar swelled in all glycocoll solutions less concentrated than 0.3 M to the amplitude attained in water and exceeded it in some cases, a fact which for the first time gives a sound basis for results in which growth was accelerated and the total increased by this compound.

Another pentosan, gum tragacanth, was dried from solutions to

form sections 0.13 mm. thick on filter paper. Swellings at 15° C. were obtained as follows:

Distilled Water.	Glycocoll. .03 *M.*	0.05 *M.*	0.01 *M.*
1380%	1382%	1077%	1462%

This gum liquefies irregularly and hence the figures show the extent of swelling before active dispersion of the mass begins.

Next a mixture of 9 parts of gelatine and 1 part tragacanth was made up at 2.5 per cent. to correspond to a similar mixture of gelatine and Opuntia mucilage. Swellings as follows at 15° C. were obtained:

Distilled Water.	Glycocoll 0.3 *M.*	0.05 *M.*	0.01 *M.*
1320%	1520%	1040%	1320%

Nothing may be concluded on the basis of these figures except that the hydration of this material reaches a stage where it goes into dispersion unevenly and in a manner which makes auxographic readings, as well as all mass or weight determinations, of doubtful value.

The above tests were repeated with Opuntia mucilage with the following results:

Distilled Water.	Glycocoll (15° C.). 0.3 *M.*	0.05 *M.*	0.01 *M.*
923%	800%	654%	600%

It is highly probable that the high relative swelling in the more concentrated solution is due to the hydrogen ion effects on the gelatine as glycocoll is known to show some dissociation in this way.

Sections consisting of 4 parts agar and 1 of gelatine which had an average thickness of .3 mm. swelled as follows at 15° C. in glycocoll.

0.3 *M.*	.05 *M.*	.01 *M.*	.002 *M.*
1550%	1233%	1960%	1767%

The average swelling of such sections in water was about 1700 per cent. and the irregularity characteristic of auxographic measurements of the action of this amino-acid is seen in the above results.

A preparation was now made in which two parts of the water soluble protein from oats was added to 8 parts of agar in a 2.5 per cent. solution of the latter. The plates dried to a thickness of .25 mm. When sections of such biocolloids were swelled in the glycocoll series, the results were as shown in the table, the hydrations in concentrations less than 0.01 M approaching and surpassing those in distilled water.

The action of acids being supposedly due to the hydrogen ion concentration, a test was made of the action of a solution in which glycocoll (amino-acetic) was added to acetic acid. Trios of surface slices of Opuntia which had dried to a thickness of 0.8 mm. swelled 163 per cent. in .05 N acetic acid and 156 per cent. in a .05 N solution of acetic acid and glycocoll. No especial significance can be attached to the lesser swelling in the double solution, except that no evidence as to acceleration of swelling by the addition of the amino-acid was obtained.

Next trios of sections of 8 parts agar and 2 parts gelatine 0.3 mm. in thickness were swelled in the acetic and amino-acetic solutions 0.01 N at 18° C. The swelling in the acetic acid alone was 1450 per cent., while that in the combined solutions was but 1300 per cent., which agreed with the previous effects in being less than in the acid alone.

Trios of sections of agar swelled 1875 per cent. in a 0.01 N solution of acetic acid at 18° C., while a combined solution of equivalent molecular concentration showed a swelling of 1750 per cent.

There now remained the test with living tissues. Some joints of *Opuntia blakeana* of 1918 which had been brought from Tucson two months earlier and had laid on the table with the result that they had lost much water but were still alive, were used for this test. A trio of sections with an average thickness of 6 mm. swelled 60 per cent. in the hundredth normal acetic acid, while a similar trio which measured 5.5 mm. on the average swelled but 45.5 per cent. in the combined acetic-glycocoll solution. A second feature distinguished the two reactions, the swelling in the acetic being continuous and approaching zero during the 20 hours of measurement, while in the combined solution full expansion was reached in 4 hours after which a shrinkage resulted in a loss of nearly 5 per cent.

A return was made to the biocolloidal mixtures and trios of sections of agar 8 parts and oat protein 2 parts with a thickness of .22 mm. were swelled at 18° C. The hydration swelling in the hundredth normal acetic acid gave an increase of 1318 per cent., while an equinormal solution of the acetic acid and glycocoll gave a swelling of 1605 per cent. This test is the only one of the series in which the addition of glycocoll to the acetic acid enhances imbibition.

An additional test was made in which equal amounts of glycocoll and acetic acid were brought together at a concentration of 0.001 *M* on agar-oat protein sections as above. The swelling in the acetic acid was 2681 per cent. or about the same as that possible in distilled water (2630 per cent.) while the swelling in the combined solution was slightly less, being 2570 per cent., a difference which is without special significance in this case, as it is near the limit of instrumental error or might have been caused by brief temperature variations.

The positive action of amino-compounds in affecting hydration is well demonstrated by the above facts. These effects are not coincident with the action of solutions offering the greatest hydrogen ion concentration, or at any point in which an optimum of such action might be assumed. The actual maximal hydrations in amino-compounds take place in reality in attenuated solutions (0.1 *M* to 0.00008 *M*), but the nature of the action has not yet been determined.

THE TEMPERATURE FACTOR IN GROWTH.

If growth were dependent in the main upon any reaction or upon a chain of consequent transformations the influence of temperature upon the rate and course might be readily and definitely established. Many authors have assumed such a state of affairs.

Growth however is an interlocking meshwork of reactions and a rise of temperature through the ordinary range from 5° C. to 30° C. may and generally does pass the point at which one or more of the reactions passes the range of its accelerating effect, and in any further rise becomes an inhibition. Such results are to be found, for example, in the action of hydrogen ions or in respiration residues. The following experiments may serve to illustrate this matter.

The petioles of some young plants of a *Solanum* hybrid in the glass house at Tucson were available on April 21, 1918. Trios of sections were placed in distilled water and acids at 18° and 38° C. with results as follows:

	Distilled Water.	Hundredth Normal Citric Acid.
18° C.	4.2%	4.2%
38° C.	11.8	2.6

The swelling in distilled water was nearly three times as great at the higher temperature, while in the acid solution a retardation took place which limited the total at the higher temperature to something over a half that possible at the lower point. The total swelling in acid at the lower temperature occupied an hour and at the higher temperature it was a matter of ten or fifteen minutes. A similar speeding up of imbibition in water was observed. The total capacity at the lower temperature was not reached for 8 or 10 hours, while at the higher it was something under 2 hours.

Plants of *Phaseolus* which formed the experimental material for measuring the growth of pods and seeds bore some pods in which the beans were nearly mature. Pods of the same stage of development as one which was under the auxograph for recording daily changes were opened and the unripe beans removed. The ends were cut away, the outer coat removed; the remainder of each cotyledon made on section of which three were taken from separate pods for swelling. The average thickness was 3.2 to 3.4 mm. and the swellings were as follows:

	Distilled Water.	Hundredth Normal Citric Acid.
38° C.	*a*....14 %	*a*....11 %
	b.... 8	*b*.... 9.7
18° C.	*a*.... 9.6	*a*.... 6.6
	b....11.7	*b*.... 4.4

The higher temperature to which *a* was subjected appears to be above the point at which maximum absorption or imbibition takes place in distilled water as the swelling was 30 per cent. less than at the point below. The retarding effect is much more marked in the acid solution, however, as the reduction of the total capacity below that shown at 18° C. amounted to 40 per cent.

The material in series *b* taken at a later date and with seeds which seemed to be more nearly mature, showed an increase in swelling in distilled water of about 45 per cent. over the total at the lower temperature, while the swelling in acid was less than half that at 18° C. The average of the two series is such that the swelling in distilled water is nearly the same at both temperatures, while in acid the average at 18° C. is 10.4 per cent., which is nearly double that at 38° C. at which point the hydration capacity seems to be invariably lower than at the lower temperature. These averages represent a total of six cotyledons each.

A final test of variations in temperature upon material in an acidified condition was made with dried sections of *Opuntia*. These sections were made by slicing away the chlorophyllous layer from one side of the flat joint and drying the remainder in the desiccator and in sheets of blotting paper in such manner that buckling and crumpling was prevented. After all of these precautions were taken, however, the measurement of the sections was subject to some error due to the fact that the fibrovascular strands remaining would increase the thickness under the calipers without reacting in due proportion to the action of the swelling agent. A wide range of figures was obtained, but it was apparent that a rise in temperature did not have an effect on material in acid equivalent to that in distilled water, as will be apparent from the following measurements obtained from sections which were .43 to .46 mm. in thickness.

	Swelling in	
	Distilled Water.	Hundredth Normal Citric Acid.
18° C.	315%	360%
	385	430
	486	460
Average	395%	417%
28° C.	453	460
38° C.	500	477
	413	400
Average	457	439

The increase in swelling in distilled water is seen to be about twice that in the acid in the rise from 18° C. to 38° C. The influence which the condition in question may exert on the rate of growth is obvious. Thus the course of enlargement of an organ or

of a cell-mass in so far as this consists in hydration may vary widely in the first instance because of the residual acids in the colloids and the balance or accumulation of this will in turn depend upon the effect of the enzymatic or respiratory processes in metabolism. Thus a rise of 10 degrees from the customary morning temperature of 15° C. which has been encountered in so many of these experiments, would result in an acceleration of growth largely determined by the state of acidosis of the plant. A rise from the same temperature later in the day or under other conditions of illumination would necessarily have a different result. An extension of the attempts to bring rates of growth into a figure or formula, therefore, would be a forced application of knowledge of one process to a complex of activities in which any change in temperature might set up opposed alterations. In consequence of this fact, the coefficient of variation for 10° C. is seen to vary from one to seven in various organisms.

The capacity for hydration and growth is a resultant of the composition and proportions of the principal components of the living matter and the relations of the phases in which they occur, modified by the "nutrient" salts absorbed in its structure, and by the products of unceasing metabolic changes, especially the transformations which are comprehended in respiration and which carry compounds through a stage in which acids are formed. These features as influenced by temperature determine the rate, daily course and total expansion in growth. In addition, a certain amount of material is lost from the plant in the form of carbon dioxid, and as has been emphasized on the preceding pages, the surface loss of water may overbalance absorption. The rate, course, and amount of growth is therefore affected by many agencies and includes multiple interlocking reactions.

Water-content, Dry Weight and Other General Considerations.

Two different types of organs or shoots with respect to the variations in the water-content and dry weight are recognizable in the material which has served for studies in growth as described in this paper and in the work of other writers. The commoner types

of woody stems, thin leaves and the organs of the greater number of the higher plants undergo a development which terminates in a mature stage in which the proportion of solid material is very much higher than that found in younger material. A parallel procedure is the prevalent one in the tissues of the higher animals. Thus by way of illustration, Donaldson found that the proportion of water in the bodies of mammals disminishes with age, and Hatai has shown that the percentage of water is an indicator of phases of chemical alteration in the composition of the body.[1]

Growth and differentiation of cell-masses into specialized tissues is not inseparably connected with increases in dry weight, however, as has been demonstrated by studies of the growth of frog larvae in the earlier stages,[2] and it is highly probable that similar phenomena are prevalent in the fleshy fungi and other lower forms of plants.

The distinction between the two kinds of growth has not been made previously in studies of plants and the matter was finally taken into consideration in the experiments late in 1918.

Etiolated plants furnish examples of growth with a small increase in proportionate dry weight, but chief interest attaches to plants which normally show such action, and the most striking illustrations are to be furnished by the organs of succulent plants and by fruits. The relative amounts of solid material in the flattened joints of Opuntia does not increase with the course of development toward maturity, and joints which have reached full size may contain over 91 per cent. of water. Secondary thickening, especially that which results from branching and the development of additional fibrovascular tissue, may cause an added amount to be formed. The proportion of dried material and water in the leaves of *Mesembryanthemum* does not vary greatly with age. These and probably all succulent forms are characterized by an exaggerated production of mucilages or pentosans, and have certain implied cycles of metabolism includ-

[1] Donaldson, H., "The Relation of Myelin to the Loss of Water in the Mammalian Nervous System with Advancing Age," *Proc. Nat. Acad. Sc.*, 2: 350. 1916.

Hatai, S., "Changes in the Composition of the Entire Body of the Albino Rat during the Life Span," *Amer. Jour. of Anat.*, 1: 23. 1917.

[2] Ostwald, W., "Ueber die zeitlichen Eigenschaften der Entwickelungsvorgänge," p. 49. 1908.

ing an incomplete type of respiration which leaves large acid residues. The total acidity of the cell-masses may vary greatly during development and during the course of a day, and the actual acidity or hydrogen ion concentration of the sap resulting from the buffer situation may also show a marked variation, but within much narrower limits.

Although the development and maturation of fruits such as berries obviously includes a growth in which the total effect is one of practical maintenance or increase in the water-content, studies of their growth seems to be lacking. It was therefore planned to arrange a final series of experiments in which the enlargement of fruits with increasing dry weight and others with low relative dry weights should be measured. The walnut was taken to represent a structure with accumulating solid matter, and the tomato for the other type.

The walnut consists of a thick fleshy exocarp and a heavy endocarp which finally becomes hard and bony with the deposition of anhydrous wall material. The enclosed embryo also accumulates a large amount of condensed food-material. The tomato is a large globose berry in which deposition and thickening is confined to the small hard seeds. The greater part of the fruit is a fleshy pulp, which becomes more highly watery as progress is made toward maturity.

Nuts of *Juglans californica* var. *quercina* Babcock, of various sizes from 3 mm. in diameter to that approaching maturity were on two trees in the garden at Carmel in June, 1918. Suitable supports being provided, the bearing lever of an auxograph was rested as lightly on the young nuts as was consistent with a clear record, and temperatures were taken by thin thermometers thrust into similar nuts or into young stems near the preparation. Fifteen nuts were measured for periods of two or three days or for as long as two months in the case of No. 10.

Coincidentally with the measurements an effort was made to determine the degree of saturation or hydration of the stems on which the nuts were borne. A well defined "negative" pressure was detected in the basal branches of *Juglans major* which was growing near the experimental tree. A basal branch 1.2 meters from the trunk gave a dry looking surface when it was cut off.

A section of a similar branch about 8 mm. in thickness and 42 cm. long was cut away from another basal branch of the tree, the end of the detached portion quickly sealed with vaseline and when all was in readiness the tip was excised and the cut thrust into water to ascertain the actual deficiency in this portion. 14 hours later a total of 6 c.c. of water had been absorbed and 24 hours later 8.5 c.c., which was a practical saturation, at a temperature of 18° to 20° C. The volume of the branch proved to be 35 c.c. so that the amount of water absorbed was 24 per cent. of the total.

Sections of young internodes of *Juglans californica quercina* which had an average diameter of about 2.5 mm. were swelled in solutions as below, then dried and swelled again with results as below at 16° C.

Water.	Citric Acid 0.01 Normal.	Potass. Hydrate 0.01 *M*.	Potass. Nitrate 0.01 *M*.
	Fresh—living.		
10%	14%	13.2%	12%
	After Drying.		
34	34	34	32
	(On basis of original thickness.)		

The unsatisfied capacity of these sections taken from young terminal internodes was comparatively great, doubtless due in part to the constant drain of the active leaves they bore. The older wood including that formed the previous year showed an absorptive capacity of 22 per cent. in water. Now it is from these older internodes that the nuts arise.

The nuts were highly turgid, exuded sap when cut into, and hence must have had a colloidal composition which acted to withdraw water from the stems which contained a much lower percentage of water. The soil was low in moisture content at this time, as it had been four to five months since the rains.

Tests of nuts 8 to 10 mm. in thickness from which tangential slices had been removed to give a uniform thickness of 7.5 mm. were made in July, and these swelled at temperature of 17 to 30° C. in solutions as follows:

Distilled Water.	Citric Acid, .01 *M*.	Potass. Hydrate, .01 *M*.	Potass. Nitrate, .01*M*.
1.5%	1.8%	1.4%	2%

A useful conception of the hydration conditions in the stems and fruits may be formed, if due weight is given to the measurements cited above. The woody branches of the previous year, on which both the leafy green twigs and those bearing the nuts are borne, had a relatively large deficiency in water so that sections a few centimeters long absorbed about 20–25 per cent. of their volume of distilled water in 24 hours at 20° C. No swelling test was made, but it is obvious that an enlargement of only a small fraction ought to be shown by this or any branch with a mature woody cylinder. The active green twigs still in a state of elongation arising from these branches had a swelling capacity of 10 per cent. The growing nuts arising from the drier stems exuded water from cut surfaces, the cotyledons being sacs of watery fluid, in contrast to the dry appearance of sections of the youngest internodes, and showed a swelling of less than 2 per cent. and soon shrunk when placed in a cylinder of distilled water after being cut in halves. In a system of this kind any alteration of the conditions which would facilitate transpiration would have a differential effect on the older stems, the green leafy twigs and the fruits. The loss from the stems would be affected least since the bark would effectually prevent any notable increase in evaporation from the relatively dry woody tissues. The loss from the leafy twigs would of course tend to become greater and the deficit in both leaves and twigs would be increased and their absorbing power correspondingly increased. The outer integument of the nuts being still in an embryonic condition and being highly hydrated the loss would reach a maximum rate with the daily effect of causing a cancellation of enlargement beginning mid-forenoon at 20–22° C. and continuing until mid-afternoon when a fall in temperature brought transpiration to a rate below that of accession from the stem.

A large percentage of the nuts which were placed under the auxograph lever were cut off at various stages of development by abscission of the stalk. The inciting causes of the actual anatomical change which cause the abscission lie outside the scope of this article. It was noted however that it was preceded by a period in which the nut showed a shrinkage by day in the higher temperatures and lessened humidity, alternating with equalizing enlargements at

nights. Finally an abrupt rapid and continuous shrinkage resulted in the separation of the stalk.

The general features of growth of these nuts may be illustrated by a résumé of history of No. 10, which was under continuous observation from July 15 to September 9, 1918, during which period of 56 days its diameter increased from 16 mm. to 26.5 mm. Of this 2.25 mm. was gained in the first five days of cool foggy weather. This effect was confirmed by the fact that a cessation or retardation occurred at midday and was most pronounced on hot sunny days, suggesting a direct water-loss. In the week ending July 29, the total growth was an increase of 1.7 mm. This period was characterized by heavy fogs and mists in the forenoon, both the amount of shrinkage and rate of increase being lessened—an equalization to be ascribed in part to approaching maturity. The temperature taken from a thermometer thrust in a young branch of the thickness of the nut ranged from 13 to 22° C. The completion of the record of No. 10 was followed by cutting of the branch bearing it at a distance of 30 cm. placing excised end in water and arranging the entire preparation in the dark room at 17° C. with the nut under the bearing lever of the auxograph. Swelling continued for about 20 hours after which shrinkage began which rapidly accelerated.

The general features of growth were also well illustrated by the following notes on No. 15, which was brought under observation when it was about 15 mm. in diameter and put under an auxograph amplifying 45 on August 3. Great daily variations in size with a net total increase were displayed every day. Actual enlargement could be detected between noon and 2 o'clock which continued until 8 or 10 the following morning, depending upon the sunshine. If the sun rose clear, shrinkage began immediately. If the morning was foggy, it would be delayed, but the increase in thickness was rapid, being mostly accomplished in 2 hours. Minor variations in this general procedure might be brought about from the shade of clouds, especially noticeable at noonday August 6 and to be seen at other times (see Fig. 1).

It is to be seen from the above that the fruit of the walnut in an environment favorable to its development exhibits daily variations in growth clearly attributable to the balance between transpiration

and absorption. The nut in a growing condition has a high water content, and a small unsatisfied capacity, but its supply from the relatively dry stems must come slowly, so slowly that any marked increase in transpiration would overbalance absorption in the nut and result in cessation of enlargement or even shrinkage.[3]

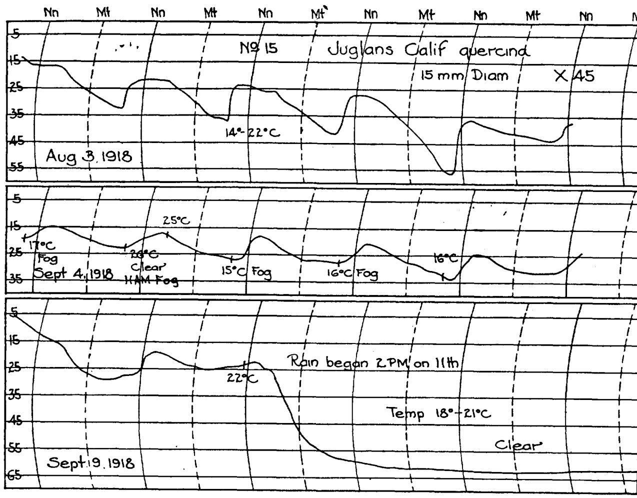

FIG. 1. Tracings of auxographic record of walnut (No. 15) during three weeks. Variations are amplified forty-five times and the downward movement of pen signifies enlargement. The scale is numbered in millimeters. Shrinkage during the midday period lessened by fogs and acceleration in growth by the humidity and increased water supply from rain are among the more striking features.

Such retardation or apparent cancellation of growth by rapid or excessive water-loss has already been discussed in connection with the presentation of my original results dealing with the growth of *Opuntia*. Recent exemplification of this action in *Cestrum nocturnum* has been described by Brown and Trelease.[4]

The fruit of the tomato (*Lycopersicum*) presents similar features of behavior. The proportion of solid matter in young fruits

[3] MacDougal, D. T., "Reversible Changes of Form in Succulents." Report Dept. Bot. Res. Carnegie Inst. of Wash. No. 14, p. 71. 1915.

[4] Brown and Trelease, "Alternate Shrinkage and Elongation of Growing Stems of *Cestrum nocturnum*," *Philip. Jour. of Science*, 13: No. 6. Bot. p. 353. November, 1918.

was greater than in those approaching maturity, representing the extreme of this type. Four fruits less than a week old, with radial diameters of 14, 16, 17 and 18 mm. were found to weigh 14.650 grams. These were fragmented and placed in a beaker on a water bath at about 100° C. for 48 hours, at which time the dry material remaining was 1.90 grams. From this it is to be seen that the young fruit contained 87 per cent. of water and 13 per cent. of dry material. A mature fruit of the same kind as those measured taken September 10 from a ranch near the Coastal Laboratory was 46 mm. in axial diameter and 58 mm. in radial diameter and weighed 93.050 grams. This was dried over a water bath for 2 days, at which time 8.400 grams remained. From this it is to be seen that the ripe fruit contained 91 per cent. of water and 9 per cent. of dry material. (See Anderson, S. P., "The Grand Period of Growth in a Fruit of *Cucurbita pepo* Determined by Weight," *Minn. Bot. Stud.*, 1: 238, 1894–98; and MacDougal "Practical Text-book of Plant Physiology, pp. 293, 294, 1918, New York.)

A number of plants of the tomato were grown in suitable boxes of soil at a ranch in the Carmel valley, and were in such a stage of development that young fruits were available at the Coastal Laboratory in August, 1918.

Six plants in all were used and continuous records from fruits of an axial diameter of 3 to 4 mm. to maturity at 50 to 55 mm. were obtained. The fruits were oblate-spheroid in form and the auxograph was arranged to register increase in diameter nearly parallel to the axis in some cases and radially or at right angles to it in others. In addition to the other advantageous features of this material their regular form and mode of growth made it possible to use the variations in diameter as a basis for calculating the changes in volume of the fruits taken as spheres.

Temperatures were taken by thrusting the thin bulbs of the small thermometers into fruits near the one under measurement. The development of such fruits was but little affected by this wounding and the thermometer remained firmly in place as in the fleshy joints of *Opuntia*, in the measurement of which this method was first practised. The preparations stood in a well-ventilated glass house and the soil around the roots was kept moist in accordance with the

cultural requirements of these plants. The results may be best set forth by the description of the action of the several fruits measured.

No. 1 was placed in the greenhouse and a fruit 29 mm. in diameter was fixed on a block of hard cork in such position that it gave a radial bearing to the auxograph which was set to amplify changes in volume by 5, on August 9. The record was kept continuously until September 18, at which time the radial diameter of the fruit was 51.5 mm. The fruit was turning yellow on September 16 and was showing fluctuations in volume comparable to those in No. 2, with which it was run in close comparison and under almost exactly the same conditions of moisture and temperature as recorded.

No. 2 was adjusted to the auxograph in the greenhouse on August 9 in such manner as to give modifications of the axial diameter, which at this time was about 27 mm. The record was continuous until September 18, at which time the diameter was 50.5 mm. This fruit like No. 1 was beginning to turn yellow on September 16.

No. 3, 10 mm. in diameter, was adjusted to the auxograph to record variations in radial diameter on August 21, and a record was kept continuously with frequent notations of temperature and sunshine, etc. It is to be noted that 1, 2 and 3 were under equable temperatures, 19 to 20° C., and high relative humidity during the rainfall of September 11 and 12.

The fact that the greatest increase in growth occurs in fruits at diameters between 16 to 25 mm. in diameter, before half the final size is reached is a point to which we shall recur in the discussion of growth in terms of volume. Thus in No. 3 the increases in thickness weekly were as follows: 6 mm., 6.3 mm., 2.5 mm., 3.5 mm.

If this method be followed it would at once be obvious that while the rate of increase in diameter would be a direct measurement yet as the fruit increases as a globe the actual material added could be regarded as a shell on this globe. The rate in terms of volume would therefore be the amount of this shell to be calculated by finding the difference between the initial volume, and the volume at the end of each period by the formula $Pi\ r — Pi\ R$ in which $r =$ the new radius and R the initial radius. The rate by direct measurement of diameter and by volume increases may be compared as below.

Average daily rate of growth measured as increase in diameter was as follows for periods beginning as follows:

Aug. 9th.	Aug. 16th.	Aug. 21st.	Aug. 28th.	Sept. 4th.	Sept. 11th.
1.7 mm.	1.1 mm.	0.7 mm.	0.4 mm.	0.28 mm.	0.17 mm.
Average daily rate of increase in volume:					
1,953 cu. mm.	1,885 cu. mm.	1,548 cu. mm.	1,036 cu. mm.	732 cu. mm.	521 cu. mm.

The rate on September 11 by direct measurement would appear to be one tenth that of a month earlier, yet actually water and new material was being added at a rate equivalent to one fourth of the earlier rate. The radial proportions would make the rate on August 21 not much more than 40 per cent. of the rate on August 9, while the increase in volume was over 96 per cent. The rate in the week beginning August 28 would appear to be less than a fourth that by direct measurement on August 9, yet actually the increment of water and material is more than half that in the younger stage and smaller size.

A second plant with the auxograph bearing arranged to take axial variations in the fruits which measured 33 mm. was arranged to run concurrently with No. 1 and under identical temperature and conditions of moisture. The daily rates of increase in diameter were as follows for the periods beginning:

Aug. 9th.	Aug. 16th.	Aug. 21st.	Aug. 28th.	Sept. 4th.	Sept. 11th.
0.95 mm.	0.7 mm.	0.56 mm.	0.3 mm.	0.2 mm.	0.2 mm.
Daily rates in terms of volume:					
1,563 cu. mm.	1,388 cu. mm.	1,274 cu. mm.	949 cu. mm.	380 cu. mm.	420 cu. mm.

Here again the actual course of growth as calculated in terms of volume shows that simple measurements of the thickness do not express the real values in growth in such organs.

The third test was made on a fruit taken at a much earlier stage at a diameter of 16 mm. with a transverse or radial bearing, the temperature and moisture conditions being similar to those of 1 and 2. The daily rate of increase was as follows for the weeks beginning on the following dates:

Aug. 21st.	Aug. 28th.	Sept. 4th.	Sept. 11.	Sept. 18th.	Sept. 25th.
.85 mm.	.85 mm.	.64 mm.	8. mm.	.3 mm.	.37 mm.
Daily rate in volume:					
537 cu. mm.	851 cu. mm.	885 cu. mm.	1,643 cu. mm.	594 cu. mm.	662 cu. mm.

The actual volume of this fruit at the close of the experiment was approximately 2,200 cu. mm. and its growth had been followed for a period of 40 days. It is notable that in the earlier stage in the advance of the fruit from 20 to 26 mm. in diameter that while the increase of the diameter seems constant yet the actual accession of material is very much greater. Then in further development the average increment to the diameter was smaller, yet the actual accession of material was greater. Following this the rate falling from 0.8 to 0.3 mm. daily the accession decreases less than half.

The record of growth of No. 3 shows beyond question the effect of transpiration and water loss on growth. As the daily temperatures of the fruits rose from 12° C. and 14° C. to 26° C. and 28° C., acceleration ensued up to a point where the rise caused a water loss overbalancing the gain by hydration. Higher temperatures therefore did not facilitate or accelerate growth unless accompanied by high relative humidity. Thus the highest are those of midday and afternoon, with fog or showers. This is especially marked on

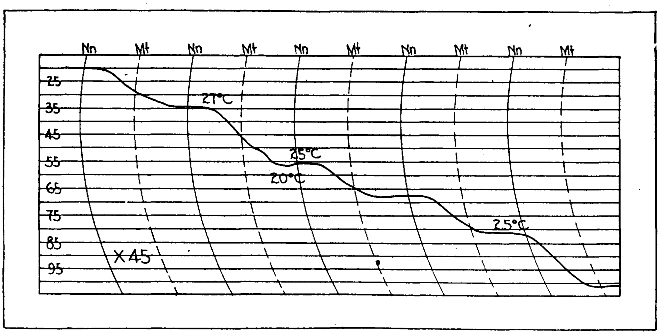

FIG. 2. Auxograph tracing of variations in volume of fruit of hybrid *solanum*. Downward course of line denotes enlargement amplified 45 times. The scale is numbered in millimeters. Midday temperatures are given.

the record of September 10, 11, 12 and 13, in which a 50-hour rainy period was anticipated and followed by high humidity. It was not possible to increase the water supply by watering the soil around the roots in such manner as to cancel the midday shrinkage or slackening in growth. One especially striking effect is that in which the rise in temperature consequent upon the cessation of the rain from

20 to 25° C. at 3 P.M. was followed by a lessened rate of growth, and on the cloudy days was uniformly high. Similar effects were exhibited by a small fruit of a potato in a greenhouse at Tucson in May, 1918.

The water deficit of the stems as measured by swelling includes that of the entire structure. The fruits however receive their supply through special conduits which sustain only a mechanical relation to the other parts of the stem which may be active in its swelling. Such non-conducting tissues of course draw their supply from this system of conduits also, but it is highly probable that the disproportion between the water content of the fruit and of the tracts in the stem from which it receives its supply is not so great as might be indicated by the measurements given. The hydration capacity of the fruits would be the resultant of many factors including the pentosan-protein ratio, the hydrogen ion concentration, the action of salts and the effect of the amino-compounds.

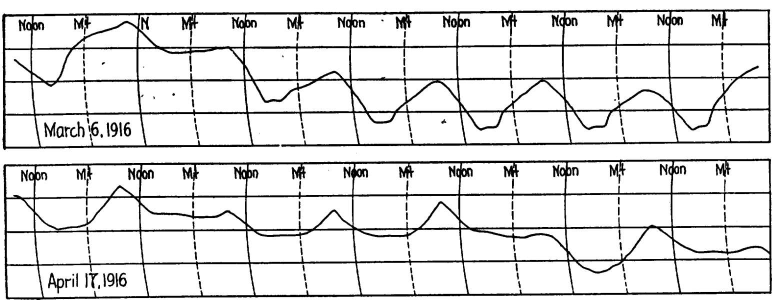

FIG. 3. Tracings of auxographic records of *Opuntia discata* during two weeks of secondary growth. Downward course of the pen denotes enlargement amplified 45 times. The scale is in centimeters. The shrinkage at night is greater in some instances than the enlargement of the preceding day.

The balance between water-loss and the gain by absorption is so delicate in the fruits the action of which was measured, and in many stems that increased humidity may be followed by accelerated growth, while a rise of ten to fifteen degrees in air temperature may check enlargement by increasing water-loss and it is also taken into account that the consequent rise in temperature of the growing stem may actually lessen the water-holding capacity of the biocolloids which make up living matter.

It would be unwise to assume, however, that the general procedure followed by the walnut, tomato and by some green stems, is universal.

The growth of the flat joints of *Opuntia* which were described before this Society in April, 1917, presents many features different to the above, and some tracings of the growth record of *Opuntia discata* for three weeks are shown in figure 3.

Enlargement (denoted by a downward course of the recording pen) begins in the morning and continues with the rising temperature until mid-afternoon, then slows down and shrinkage sets in which continues through the night. Such shrinkage must inevitably accompany and result from excessive water-loss, and in confirmation it is found that the cacti show the greatest transpiration at night, at which time the acidity rises until it is ten times as great as in the daytime. The disintegration of this by light and higher temperatures increases the imbibition capacity of the cells known to be high in pentosans and a swelling or growth in the daytime results, producing a growth record almost exactly in reverse of that of the walnut and tomato.

The rate, course and amount of growth are at all times a resultant of agencies which affect water-loss, hydration capacity, respiration and its residues and other features of metabolism.

The procedure in transpiration from the surfaces of a growing organ may be such that the maximum loss partly masking growth, may for example occur in the cacti at night, while it comes in the midday period in the commoner types. The swelling or hydration capacity of any plasmatic mass which determines its capacity for growth or enlargement depends in the main on the mucilages or pentosans present and their amount relative to the proteins, the character of which also affects growth.

The biocolloids of the plant show a degree of swelling in water greater than that in solutions containing free hydrogen ions, so that growth generally is most rapid in cell solutions near the neutral point. In modification of this last statement, it is to be pointed out that maximum swelling effects may result from the action of some of the amino-acids.

Rises in temperature within the range ordinarily associated with

growth may result in lessened swellings or hydrations, a result to be connected directly with certain fluctuations in the growth rate.

Succulent stems, leaves and fruits may show growth in which development or age is not accompanied by an increase of relative dry weight. In some of these structures, such as the tomato, it is possible to analyze the auxographic record and determine the actual total accretion of material in any stage of development. The graph plotted from such data does not follow the contours of a figure plotted from the variations in thickness or diameter, the chief difference being that the maximum comes at a later period.